Combination of sodium caseinate and succinylated alginate improved stability of high fat fish oil-in-water emulsions

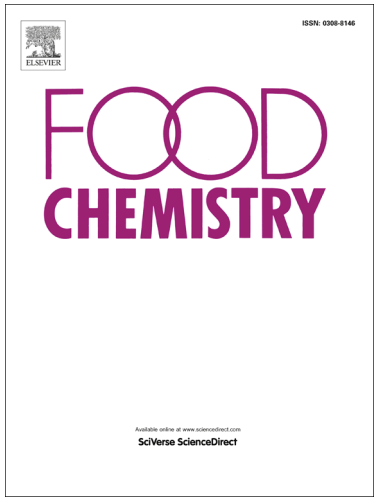
Yesiltas, Betül; Sørensen, Ann-Dorit Moltke; García Moreno, Pedro Jesús; Anankanbil, Sampson; Guo, Zheng; Jacobsen, Charlotte

Published in:
Food Chemistry

Link to article, DOI:
10.1016/j.foodchem.2018.02.074

Publication date:
2018

Document Version
Peer reviewed version



Combination of sodium caseinate and succinylated alginate improved stability of high fat fish oil-in-water emulsions

Betül Yesiltas, Ann-Dorit Moltke Sørensen, Pedro J. García-Moreno, Sampson Anankanbil, Zheng Guo, Charlotte Jacobsen

PII: S0308-8146(18)30312-1
DOI: https://doi.org/10.1016/j.foodchem.2018.02.074
Reference: FOCH 22458

To appear in: *Food Chemistry*

Received Date: 4 August 2017
Revised Date: 27 January 2018
Accepted Date: 13 February 2018

Please cite this article as: Yesiltas, B., Sørensen, A.M., García-Moreno, P.J., Anankanbil, S., Guo, Z., Jacobsen, C., Combination of sodium caseinate and succinylated alginate improved stability of high fat fish oil-in-water emulsions, *Food Chemistry* (2018), doi: https://doi.org/10.1016/j.foodchem.2018.02.074

Combination of sodium caseinate and succinylated alginate improved stability of high fat fish oil-in-water emulsions

Betül Yesiltas[a], Ann-Dorit Moltke Sørensen[a], Pedro J. García-Moreno[a], Sampson Anankanbil[b], Zheng Guo[b], Charlotte Jacobsen[a]*

betye@food.dtu.dk, adms@food.dtu.dk, pejeg@food.dtu.dk, sampsonanankanbil@eng.au.dk, guo@eng.au.dk, chja@food.dtu.dk

[a]Division of Food Technology, National Food Institute, Technical University of Denmark, Denmark

[b]Division of Lipid Biotechnology and Engineering, Department of Engineering, Aarhus University, Denmark

*Correspondence: Charlotte Jacobsen, National Food Institute, Technical University of Denmark, Kemitorvet, Building 204, 2800 Kgs. Lyngby, Tel: +45 23 27 90 75; e-mail: chja@food.dtu.dk

Highlights

- Fish oil content and type of emulsifier affected stability of fish o/w emulsions
- Addition of alginate improved stability of fish o/w emulsions stabilized with casein
- Long chain modified alginate showed better emulsifying capacity
- Short chain modified alginate improved physical and oxidative stability of emulsions

Abstract

Sodium caseinate (CAS) and commercial sodium alginate (CA), long chain modified alginate (LCMA) or short chain modified alginate (SCMA) were used in combination for emulsifying and stabilizing high fat (50-70%) fish oil-in-water emulsions. Physical (creaming, droplet size, viscosity and protein determination) and oxidative (primary and secondary oxidation products) stabilities of the emulsions were studied during 12 days of storage. Creaming stability was higher for emulsions produced with alginates and CAS compared to emulsions prepared with only CAS. Combined use of CAS+LCMA performed better in terms of physical stability compared to emulsions produced with only CAS. However, the oxidative stability of this emulsion was inferior probably due to the presence of an unsaturated carbon chain in LCMA structure. CAS+SCMA emulsions not only showed better physical stability such as smaller droplet size, lower creaming and higher viscosity, but also had an improved oxidative stability than emulsions produced with only CAS.

Keywords

Lipid oxidation, omega-3, 50-70% o/w emulsion, emulsifier, modified alginate, physical stability, oxidative stability, cod liver oil

1. Introduction

Long chain (LC) omega-3 polyunsaturated fatty acids (PUFA) have been found to decrease cardiovascular diseases and improve immune system and mental health (Song, Shieh, Wu, Kalueff, Gaikwad & Su, 2016; Wysoczański et al., 2016; Nichols, McManus, Krail, Sinclair & Miller, 2014). LC omega-3 PUFAs such as eicosapentaenoic acid (EPA, 20:5) and

docosahexaenoic (DHA, 22:6) can only be synthesized from alpha linolenic acid in the human body with a limited conversion rate. As fish oil is a good source for LC omega-3 PUFAs, efforts have been made to enrich food products with fish oil to reach the recommended daily intake levels of LC omega-3 PUFAs (EFSA Panel on dietetic Products, 2010).

Oil-in-water emulsions are often used as a base for delivering bioactive compounds such as omega-3 PUFAs by enhancing their solubility and oxidative stability in food systems (McClements, Decker & Weiss, 2007). Many studies have been carried out evaluating low fat (up to 30%) fish oil-in-water emulsions for enriching food systems with omega-3 PUFAs (Let, Jacobsen & Meyer, 2007; Berton-Carabin, Ropers & Genot, 2014; García-Moreno, Guadix, Guadix & Jacobsen, 2016). However, to the best of the authors' knowledge, only a few studies focusing on the stabilization of high fat (>50%) fish oil-in-water emulsions have been reported (Horn et al., 2011; Horn, Nielsen, Jensen, Horsewell & Jacobsen, 2012; Yesiltas, García-Moreno, Sørensen & Jacobsen, 2017). High fat fish oil-in-water delivery emulsions provide advantages when enriching foods with omega-3 PUFAs due to their higher fish oil content compared to low fat emulsions, which means that lower amounts of emulsions would be required for enrichment. This allows minimum modification of the original recipe and makes the enrichment process easier, especially for foods containing high amounts of fat such as mayonnaise, cream cheese and dressings.

Nevertheless, both physical and oxidative stability challenges arise in high fat fish oil-in-water emulsions, which might differ from low fat emulsions. Hadnadev, Dokic, Krstonosic & Hadnadev (2013) reported that increasing oil content might result in larger droplet sizes at lower emulsifier concentration and less intense homogenization. Thus, it might make the high

fat emulsions more prone to creaming, although they normally have higher viscosity compared to low fat emulsions (e.g. due to the higher ratio oil:water). Moreover, high concentration of dispersed phase leads to an emulsion packed with droplets and thereby larger total interfacial area. This might favor oxidation of omega-3 PUFA as oxidation is claimed to be initiated at the interface and then propagated to the lipid phase (Jacobsen, Adler-Nissen & Meyer, 1999; McClements & Decker, 2000; Sørensen, Baron, Let, Brüggemann, Pedersen & Jacobsen, 2007). Lipid oxidation occurs due to the high content of fish oil rich in omega-3 PUFAs which makes the emulsion susceptible to oxidation.

Physical and oxidative stability are commonly achieved by using emulsifiers and stabilizers, which have good emulsifying and stabilizing properties as well as antioxidant activity. Hence, selection of appropriate emulsifiers is crucial for the final physical and oxidative stability of the emulsion. Sodium caseinate (CAS) and sodium alginate (CA) are commercial emulsifier and stabilizer, respectively; which have previously been reported to be used in combination in oil-in-water emulsions (Pallandre, Decker & McClements, 2007; Sosa-Herrera, Lozano-Esquivel, Ponce de León-Ramírez & Martínez-Padilla, 2012). A previous study in our lab showed that high fat emulsions prepared at pH 7 and stabilized with CAS were more oxidatively stable compared to other emulsions stabilized with phospholipid based emulsifiers and to neat fish oil (Horn, Nielsen, Andersen, Søgaard, Horsewell & Jacobsen, 2011). This is mainly explained by the metal chelating activity of CAS and to its flexible structure, which allowed a better coverage of the droplets (Pallandre et al., 2007; Sosa-Herrera et al., 2012; Berton-Carabin et al., 2014). Furthermore, our recent study indicated that combinations of CAS and CA (used as thickening agent) successfully stabilized high fat (50-70%, w/w) fish oil-in-water

emulsions both in terms of physical and oxidative stability, but it would still be an advantage if oxidative stability could be further improved (Yesiltas et al., 2017).

Alginates have recently been modified with succinic anhydride in order to increase their antioxidant properties and make them surface active (Falkeborg et al., 2014; Falkeborg and Guo, 2015). For instance, Falkeborg and Guo (2015) reported that 30% oil-in-water emulsions stabilized with 3% of alginate modified with dodecenyl succinic anhydride (SAC12) had lower creaming and higher oxidative stability compared to emulsions stabilized with β-lactoglobulin or CA. This was attributed to: i) a reduction in droplet size as a result of the improved interfacial properties of the modified alginate, ii) an improved physical barrier at the oil-water interface due to the presence of the modified alginate, which protect the lipid from pro-oxidants, and iii) an enhanced radical scavenging and metal chelating properties of the modified alginates in emulsions due to the additional carboxyl groups originating from modification as well as bringing the antioxidant active sites of the molecule close to the interface. Therefore, considering these properties, it was hypothesized that the combined use of CAS and CA/modified alginates would provide better physical stability compared to emulsions produced only with CAS by decreasing the droplet size and creaming. Moreover, modified alginates, which had improved emulsifying abilities and antioxidant activities, were expected to locate at the water-oil interface, thereby providing better oxidative stability compared to CA in high fat fish oil-in-water emulsions. In addition, the chain length of the modified alginates is expected to influence their location in emulsions and, thereby their physical and oxidative stabilities. Scientific significance of employing modified stabilizers/emulsifiers in improving the stability of oil-in-water emulsions is to understand the

behaviors of these modified compounds which might provide interfacial engineering solutions for satisfying the needs of food industry for producing health promoting food products.

In light of the above, this study aimed to improve physical and oxidative stability of high fat oil-in-water omega-3 delivery emulsions by evaluating the combined use of CAS and modified alginates. Three types of alginates with hypothetically different interfacial and antioxidant properties were assayed in combination with CAS: a) commercially available sodium alginate (commercial alginate – CA), b) alginate modified with succinic anhydride (SAC0) (short chain modified alginate – SCMA), and c) alginate modified with dodecenyl succinic anhydride (SAC12) (long chain modified alginate – LCMA). Particularly, the effect of oil content (50, 60 and 70%, w/w) as well as the combination of emulsifiers/stabilizers (CAS, CAS+CA, CAS+LCMA, CAS+SCMA) were evaluated with respect to the physical (creaming, droplet size, viscosity and protein determination) and oxidative stability (primary and volatile secondary oxidation products) of high-fat fish oil-in-water emulsions.

2. Materials and methods

2.1. Materials

Cod liver oil was provided by Maritex A/S, subsidiary of TINE, BA (Sortland, Norway), and stored at -40°C until use. Peroxide value was 0.12± 0.08 meq peroxide/kg oil. Alpha-, gamma- and delta-tocopherol results were reported as 250±1.9, 118± 1.2 and 48±0.9 µg/g cod liver oil, respectively. Sodium caseinate (Miprodan 30) was kindly donated by Arla Foods Ingredients amba (Viby J, Denmark). Arla reported a protein content of 92% in sodium caseinate for Miprodan 30. Commercial sodium alginate (Grindsted® Alginate FD 170) was

provided by DuPont (Brabrand, Denmark). Modified alginates SAC0 (SCMA) and SAC12 (LCMA) were produced according to the method described by Falkeborg, Paitaida, Shu, Pérez & Guo (2015). SCMA was produced by modifying commercial sodium alginate with SAC0 and LCMA was produced by modifying commercial sodium alginate with SAC12 which includes an unsaturated double bond (see the supplementary material for chemical structures of both modified alginates). The degree of succinylation of SCMA and LCMA were 28.63±0.02% and 35.30±0.01%, respectively.

2.2. Emulsion preparation and sampling

Aqueous phase of emulsions were prepared by dissolving both emulsifiers in distilled water and left overnight on a stirrer at 4°C and the day after pH was adjusted to 7.0 with 2M HCl or 2M NaOH. Emulsions were produced in 500 g batches in a Stephan Universal mixer (Stephan, UMC5, Hameln, Germany) equipped with an emulsification blade as described by Horn et al. (2011). Fish oil concentration with 3 different levels (50, 60 and 70%, w/w) and combination of emulsifiers with 4 different types (CAS+LCMA, CAS+SCMA, CAS+CA and only CAS) were the two factors set for the experimental design. According to our previous study, 1.4% (w/w) total emulsifier and 1.2:1 ratio CAS:CA were found to be the optimum values for stabilizing high fat emulsions using combinations of CAS and CA (Yesiltas et al., 2017). Thus, these were the values used in this study for total amount of CAS+alginate and ratio CAS:alginate. Twelve emulsions were produced (Table 1). After emulsions were produced, Fe^{2+} (0.03‰ $FeSO_4 \cdot 7H_2O$ solubilized in water, corresponding to approximately 100 µm in the final emulsion) and 0.05% sodium azide were added into the emulsions in order to accelerate oxidation and prevent microbial growth, respectively. Each emulsion was

divided in portions of 85 g, which were stored in 100 mL glass bottles at room temperature in darkness for 12 days and samples were taken on days 0, 2, 5, 8 and 12. Creaming index was measured on days 1, 5, 9 and 12. Droplet size and viscosity of emulsions were measured on days 1 and 12. Protein content in the aqueous phase was measured using frozen samples from day 5. Peroxide value, tocopherols and volatile compounds were analyzed on days 0, 2, 5, 8 and 12. Samples for lipid oxidation analysis were kept at -40°C until analysis.

2.3. Characterization of emulsions

2.3.1. Creaming index

Creaming of emulsion samples were followed in 100 ml storage bottles. Creaming index was calculated by measuring the height of total emulsion (a) and height of water phase separated in the bottom of the bottle (b); following that (b) divided by (a) and multiplied by 100. This gave the percentage of the creaming of the emulsion sample on a specific sampling point and this was calculated for all sampling points without replicates.

2.3.2. Droplet size

Droplet size of the emulsions was measured by laser diffraction (Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK) using the method described by Let et al. (2007) and Horn et al. (2011). Results were given as the volume weighted mean diameter $D[4,3]=\Sigma n_i d_i^4/\Sigma n_i d_i^3$. Measurements were carried out in duplicates.

2.3.3. Apparent viscosity

Viscosity was measured using a stress-controlled rheometer (Stresstech, Reologica Instruments AB, Lund, Sweden) equipped with a CC25 standard bob cup system in a temperature vessel. Measurements were done at 25°C over a shear stress range from 0.0125 to 400 Pa. Apparent viscosity results were obtained at a shear rate of 20 s^{-1} and expressed in Pa·s. Viscosities were measured twice on each emulsion.

2.3.4. Relative protein content in the aqueous phase

Protein determination in the aqueous phase of emulsions was done mainly based on the method described by Jacobsen, Meyer & Adler-Nissen (1998). Emulsions (~20 g) were centrifuged for 10 min at 25,400 g and 10°C (Sorvall RC-6 PLUS, Thermo Fisher Scientific, Osterode, Germany; rotor SS-34) and the oil phase was removed by the use of a pipette. The rest was mixed with distilled water (1:2) and then subjected to ultracentrifugation (Beckman Ultracentrifuge L8-60M, Fullerton, CA; rotor 21102) for 16 h at 106,979 g and 15°C, and once again the aqueous phase was extracted by the use of a syringe. The aqueous phase was diluted 1:6 in distilled water and protein concentration was determined using a BCA protein assay reagent kit (Pierce, Thermo Scientific, Rockford, IL, USA) by measuring in a spectrophotometer (Shimadzu, UV mini 1240, Kyoto, Japan) at 562 nm. Results were presented as relative protein content in the aqueous phase which was obtained by calculating the percentage of protein content in the aqueous phase compared to total protein amount added into the emulsion.

2.4. Lipid oxidation in emulsions

2.4.1. Primary oxidation products – peroxide value (PV)

For determination of primary oxidation products, a lipid extract was prepared according to the method described by Bligh and Dyer (1959) using 5 g of emulsion for each extraction and a reduced amount of solvent (60 mL of methanol and chloroform, 1:1). PV was subsequently measured on the lipid extracts by colorimetric determination of iron thiocyanate on a spectrophotometer (Shimadzu, UV mini 1240, Kyoto, Japan) at 500 nm, as described by Shantha and Decker (1994). Measurements were carried out in duplicate.

2.4.2. Tocopherol content - HPLC

Tocopherol contents of emulsions were determined by HPLC (Agilent 1100 Series; Column: Waters Spherisorb 3 µm Silica; 4.6 x150 mm) using lipid extracts (see section 2.4.1) which were further evaporated and re-dissolved in heptane. Tocopherol analyses were carried out according to the official AOCS method (AOCS, 1998) in duplicates.

2.4.3. Secondary volatile oxidation products – dynamic headspace (DHS) GC-MS

Secondary volatile oxidation products were analyzed according to the method described by Jacobsen et al (1999). Approximately 4 g of emulsion was mixed with 2 mL antifoam and 10 mL distilled water in a 100 mL purge bottle. The bottle was heated in a water bath at 60°C while purging with nitrogen (flow 150 mL/min, 30 min). Volatile compounds were trapped on Tenax GR tubes and separated in a gas chromatograph (Agilent Technologies, 6890N Network GC System) on a 30 m DB 1701 fused silica capillary column (0.25 mm i.d., 1 µm film thickness; Agilent Technologies, J&W GC Columns, USA). The oven program had an initial temperature of 45°C for 5 min, increasing with 1.5 °C/min until 55°C, with 2.5°C/min until 90°C, and with 12.0°C/min until 220°C, where the temperature was held for 4 min. The

individual volatile compounds were analyzed by mass spectrometry (Agilent 5973 Network Mass Selective Detector, Agilent Technologies, electron ionization mode, 70 eV; mass to charge ratio scan between 30 and 250) and identified by MS-library searches (Wiley 138 K, John Wiley and Sons, Hewlett-Packard). Quantification was done through calibration curves where the external standards employed were 2-ethyl-furan, 1-penten-3-one, 1-penten-3-ol, (*E*)-2-pentenal, hexanal, (*E*)-2-hexenal, (*Z*)- 4-heptenal, 2-pentyl-furan, (*E*)-2-heptenal, benzaldehyde, (*E,E*)-2,4-heptadienal, nonanal, (*E,Z*)-2,6-nonadienal. Measurements were made in triplicate for each sample.

2.5. Statistical analyses

Statgraphics XVII (Statpoint Technologies, Inc., Virginia, USA) was used to carry out the statistical analysis. Multifactor analysis of variance (ANOVA) was performed followed by Fisher's least significant difference. The significance of all terms in the models was judged statistically by computing the p-value at a confidence level 1-α = 95%. Principal component analysis (PCA) was done by Latentix 2.12 (LatentiX, Copenhagen, Denmark). The PCA was carried out with the emulsions as objects and creaming, viscosity, droplet size, oil content, protein content in the aqueous phase, peroxide value and volatile compounds as variables. Data set were autoscaled to make the variables contribute equally to the model.

3. Results and discussion

3.1. Characterization of emulsions

3.1.1. Relative protein content in the aqueous phase

Relative protein content in the aqueous phase decreased significantly ($p<0.05$) with increasing fish oil content from 50% to 70% for the emulsions produced with CAS+CA (added CAS was 0.76% of the total emulsion) (Fig. 1a). This indicated that more CAS was adsorbed at the oil-water interface when the fish oil content increased; which correlated well with the larger interfacial area obtained (D[3,2] of 5.25±0.12 and 2.07±0.20 μm for CAS+CA 50% and CAS+CA 70%, respectively) as a consequence of the higher oil content and smaller droplet sizes. Moreover, the relative protein content in the aqueous phase of CAS+LCMA-70% (31%) or CAS+SCMA-70% (25%) was higher compared to CAS+CA-70% (12%); thereby it led us to conclude that LCMA and SCMA were better incorporated at the interface (i.e. replacing CAS) compared to CA. This finding was in line with the study carried out by Falkeborg and Guo (2015), who pointed out that CA tends to locate at the water phase instead of being adsorbed at the oil-water interface.

3.1.2. Droplet size

Droplet size of the emulsions did not show any significant increase during storage; therefore only data from day 1 was shown (Fig. 1b). Droplet size decreased significantly ($p<0.05$) with the increasing fish oil content from 50% to 70% for all emulsions independently of the combination of emulsifiers used. This could be due to the relative increase in the emulsifier content in the aqueous phase when the oil content in the emulsion increased and more concentrated water phase was obtained, which in the end directly affects the viscosity of the final emulsion (Tesch & Schubert, 2002; Aken, 2006). The mechanism might work as follows: high viscosity of the water phase might allow better disruption of oil droplets by Stephan

mixer. Moreover, having higher oil fraction in an emulsion also increases the viscosity of the mixture inside the Stephan mixer, which may also enhance the disruption of oil droplets.

Droplet size increased significantly depending on the emulsifiers employed in the following order CAS+LCMA<CAS+CA<CAS+SCMA<CAS. Falkeborg and Guo (2015) also found that 30% oil-in-water emulsions produced by stirring using only LCMA as emulsifier had significantly smaller droplets (7.64 μm) compared to emulsions stabilized only with CA (22.02 μm), which indicated the superior emulsifying properties of LCMA. Emulsions prepared with only CAS had significantly bigger droplet sizes compared to the rest of the emulsions, which indicates that combining commercial or modified alginates with CAS contributed to obtaining smaller droplet sizes. As commented above, this might be due to the increase in viscosity of the water phase in the presence of CAS and alginates together, which allowed more efficient disruption of the oil in small droplets in the Stephan mixer. Moreover, emulsions prepared with CAS+LCMA had significantly smaller droplets than CAS+SCMA, which indicated that modifying CA with a long fatty acid chain led to a faster adsorption at the water-oil interface during homogenization and to the stabilization of more oil droplets before they coalesced. These results were in agreement with the significantly higher protein content in the water phase of emulsions prepared with CAS+LCMA compared to CAS+SCMA (Fig. 1a), which indicated that more LCMA adsorbed at the water-oil interface compared to SCMA. LCMA had a slightly higher modification degree (35.3%) than the SCMA (28.6%). The modification degree may also impact the differences observed in droplet size between LCMA and SCMA due to the increase in surface activity with increased modification. However, the impact of the modification degree has to be further evaluated.

On the contrary, even though SCMA showed better emulsifying capacity compared to CA (according to results from protein content in the aqueous phase); the emulsions stabilized with CAS+CA had smaller droplets than the emulsion stabilized with CAS+SCMA. The latter correlated well with the higher viscosity of the emulsion stabilized with CAS+CA (Fig. 1c); which permitted a more severe disruption of the oil in droplets in the homogenizer employed. In any case, these findings suggest that CA and SCMA had different mechanisms for physically stabilizing the emulsions; CA worked as a stabilizer whereas SCMA worked as an emulsifier in the emulsion system.

3.1.3. Apparent viscosity

All emulsions showed shear thinning behavior. This is in line with another study which reported that the addition of xanthan gum to 20% v/v menhaden oil-in-water emulsions emulsified with whey protein isolate also led to shear thinning behavior (Sun, Gunasekaran & Richards, 2007). Apparent viscosity of the emulsions at 20 s^{-1} shear rate did not change significantly during storage except for CAS+LCMA-70% (from 11.59±0.04 to 10.83±0.11 Pa·s), CAS+LCMA-50% (from 0.26±0.01 to 0.20±0.00 Pa·s) and CAS+SCMA-50% (from 0.28±0.01 to 0.17±0.03 Pa·s), which significantly decreased after 12 days of storage. Therefore, data was shown only for day 1 in Fig. 1c. CAS+LCMA-50% and CAS+SCMA-50%, which had significant decrease in their viscosity, contained 50% fish oil and had creaming during storage (Fig. 2). However, even though CAS+LCMA-70% had a significant decrease in its viscosity, it still had the highest viscosity value within all emulsion samples with no creaming during 12 days of storage (Fig. 2).

Results indicated that both fish oil content and emulsifier types had a significant effect on viscosity ($p<0.05$). It was observed that the viscosity of the emulsions became higher with increasing fish oil content; whereas the droplet sizes became smaller (Section 3.1.2). This could be due to the high concentration of dispersed phase which leads to an emulsion packed with droplets i.e. close packing of emulsion droplets and thereby larger total interfacial area. This negative correlation between droplet size and viscosity might also be explained by the increased friction forces between smaller droplets caused by expanded surface-to-volume ratio of the dispersed phase, which also results in less mobility in the emulsion and therefore higher viscosity compared to having bigger droplets (Pal, 1996). Moreover, relative increase of the emulsifier content in the water phase, due to increasing oil phase at a fixed emulsifier concentration, promotes increased viscosity of the water phase. This increase in the viscosity of the water phase leads to high shear forces and results in higher viscosity of final emulsion as explained in detail under the section 3.1.2.

Alginates are known for their thickening properties, which increase the viscosity of the products they are incorporated into (Antonov, Van Puyvelde & Moldenaers, 2004; Chen, McClements & Decker, 2010). Fig. 1c also shows that substitution of some of the CAS with different alginates increased the viscosity of the emulsions significantly for all fish oil concentrations (50, 60 and 70%). For 60% and 70% fish oil contents, viscosity of the emulsions was higher when LCMA was used followed by CA and SCMA.

3.1.4. Creaming index

Creaming decreased with increasing oil content for all emulsions, which had the same total emulsifier content, but different emulsifier combinations (Fig. 2). Emulsions produced with 50% fish oil showed 10-33% creaming at the last day of the storage, whereas emulsions produced with 70% fish oil did not have any creaming except for emulsion produced with only CAS (5% creaming). Increased creaming for 50% oil-in-water emulsions compared to 70% emulsions was supported by the fact that gravitational separation increases with decreasing droplet concentration. It has a direct link to concentration of dispersed phase, since the movement of a droplet is prevented by the surrounding droplets (McClements et al., 2007). Mayonnaise was shown as a good example for retarding gravitational separation where the droplet concentration is high (e.g. 80% fat content) (McClements et al., 2007). Sun and Gunasekaran (2009) also reported that increasing oil phase volume fraction played an important role on decreasing creaming and increasing viscosity in menhaden oil-in-water emulsions emulsified with combined use of 1 or 2 wt% whey protein isolate and 0.2 wt% xanthan gum when the oil fraction increased from 20 to 40% v/v. Likewise, an increase in oil concentration (5-40%, w/w oil) led to a decrease in creaming independently of examined different triethanolamine oleate concentrations (3-20%) and homogenization time ranges (5-60 min), which was due to an increase in packing density and inter droplet interactions (Hadnadev et al., 2013).

Since emulsions with 70% fish oil stabilized with combinations of CAS and LCMA/SCMA/CA did not show creaming, the finding confirmed the contribution of alginates on retarding creaming and improving physical stability of emulsions. Additionally, emulsions prepared with only CAS had lower viscosity compared to the rest using combinations of CAS and

LCMA/CA/SCMA (Section 3.1.3). The most stable emulsions against creaming were produced with CAS+CA which was due to CA`s tendency to locate in the water phase instead of being located at the interface, which hindered droplets movement (Falkeborg and Guo, 2015). All emulsions with 50% fish oil content had creaming; however, results were different depending on the emulsifier types.

3.2. Lipid oxidation measurements of emulsions

3.2.1. Primary oxidation products – peroxide value (PV)

Primary oxidation products increased significantly between days 0, 2 and 5; however, there was no significant increase between days 5, 8 and 12 (Fig. 3). Emulsions prepared with 70% of fish oil oxidized significantly more than emulsions prepared with 60% and 50% fish oil. High oil concentration led to lower oxidative stability, which contradicts the results found in some other studies (Osborn & Akoh, 2004; Sun & Gunasekaran, 2009; Berton-Carabin et al., 2014). However, it is important to point out that all the 50% emulsions and some of the 60% emulsions had creaming during their storage. First of all, creaming led to oil droplets being in contact with a lower amount of water phase as the droplets accumulated on the top part of the bottle and most of the water phase stayed at the bottom. Secondly, creamed droplets were closely packed, therefore some parts of the interface were in contact with other droplets`s interface instead of water phase. Thereby, creaming limited the interaction between prooxidants (e.g. traces of iron) present in the aqueous phase and lipids in these emulsions.

Emulsifier type also had a significant effect on formation of primary oxidation products, as also reported by Fomuso, Corredig & Akoh (2002) where lecithin, Tween 20, whey protein

isolate, mono-/diacylglycerols, and sucrose fatty acid ester were investigated in two different concentrations (0.25 and 1%) in 10% structured lipid oil-in-water emulsions. CAS+LCMA-60%, CAS+CA-70% and CAS+LCMA-70% were oxidized significantly more than the rest of the emulsions (<4.4 meq peroxide/ kg oil) having peroxide values of 11.6, 9.0 and 7.9 meq peroxide/ kg oil, respectively (Fig. 3). Emulsions produced with CAS+LCMA had significantly higher PVs compared to the rest of the emulsions; this could be due to the unsaturated nature of LCMA (Falkeborg et al., 2015). Emulsions produced with CAS+CA had significantly higher PVs than emulsions produced with CAS+SCMA and only CAS. This might be due to the lower content of CAS in the aqueous phase of CAS+CA emulsions compared to CAS+SCMA or CAS emulsions (Fig. 1a); since CAS has metal chelating activity and traps metal ions present in the aqueous phase (Gallaher, Hollender, Peterson, Roberts & Coupland, 2005; Horn et al., 2012). CAS+SCMA emulsions had similar PVs as CAS emulsions and showed improved physical stability such as less creaming and higher viscosity. Moreover, better antioxidative properties of SCMA than CA and LCMA might be due to the presence of a terminal carboxyl group in its structure (Falkeborg et al., 2015), which may be involved in chelation of metal ions both at the water-oil interface and aqueous phase (Hudson, 1990). Additionally, protein determination results showed that the amount of CAS dissolved in the water phase was higher for the CAS+LCMA emulsions compared to CAS+SCMA emulsions (Fig. 1a), which indicates that SCMA was present in higher concentration in the water phase than LCMA. Hence, SCMA was expected to behave as a better metal chelator in emulsions due to its preferable location in the aqueous phase.

3.2.2. Tocopherol content

Alpha-, gamma- and delta-tocopherols were quantified on day 0 to be in the range of 220.9±1.8 - 232.1±2.5, 41.6±0.7 - 43.7±1.5 and 100.9±2.0 - 106.6±4.0 mg/kg emulsion, respectively. There were no significant decrease in delta-tocopherol and gamma-tocopherol during storage for all emulsion samples. Emulsions produced with CAS+LCMA-50, 60 and 70% as well as CAS+SCMA-60 and 70% showed a significant decrease in alpha-tocopherol content during 12 days storage (see supplementary material). This indicated that the formation of primary and secondary oxidation products during storage was reduced by the antioxidant activity of alpha-tocopherol. Even though the consumption of alpha-tocopherol showed that it acted as an antioxidant in these emulsions and prevented some of the oxidation, CAS+LCMA emulsions still had higher oxidation compared to CAS+SCMA-60 and 70% which did not show high PV values (section 3.2.1). Emulsions produced with only CAS or CAS+CA did not show any significant decrease in their alpha-tocopherol content during their storage.

3.2.3. Secondary oxidation products – dynamic head space (DHS) GC-MS

Fig. 4 shows the content of selected volatiles, namely 1-penten-3-ol, (*Z*)-4-heptenal, (*E,E*)-2,4-heptadienal and (*E,Z*)-2,6-nonadienal during storage. These compounds were chosen due to their higher concentration compared to others, as well as being representative for the rest of the volatile compounds except for nonanal which was not identified for CAS+LCMA samples. Moreover, these 4 volatile compounds are secondary oxidation products formed from oxidation of omega-3 PUFAs such as EPA and DHA (Hernandez, 2011). It was observed that the emulsions produced with CAS+LCMA were separated from the other emulsions on the 2nd day of storage by having higher concentrations of volatile compounds

(Fig. 4). Emulsions CAS+LCMA-70% and CAS+LCMA-60% formed significantly higher concentrations of 1-penten-3-ol, (*Z*)-4-heptenal, (*E,E*)-2,4-heptadienal and (*E,Z*)-2,6-nonadienal compared to CAS+SCMA and only CAS emulsions in all levels of fish oil content on day 12. As emulsions produced with alginates and CAS have a negative surface charge due to sodium caseinate is negatively charged above its isoelectric point (pH 4.7-5.2) (Swaisgood, 1992) and both modified and commercial alginates are negatively charged due to carboxylate groups in their structure (Aken, 2006; Falkeborg et al., 2015); metal ions are expected to move towards water-oil interface due to attractive electrostatic forces. Although some of them could be chelated by carboxyl groups of LCMA or amino acid residues of CAS (Hudson, 1990; Tong, Sasaki, McClements & Decker, 2000; Faraji, McClements & Decker, 2004), the non-chelated metal ions could interact with the double bond which is located on the long carbon chain part of LCMA (Falkeborg et al., 2015). This is not the case for emulsions stabilized with CA and SCMA, since these alginates do not present any double bond in their structure. Additionally, CAS+LCMA emulsions had the smallest droplet sizes compared to other emulsions (Fig. 1b) and, therefore, highest interfacial area which might have favored initiation of lipid oxidation chain reactions (Jacobsen, Adler-Nissen & Meyer, 1999).

CAS+CA-70% had significantly higher formation of 1-penten-3-ol and (*Z*)-4-heptenal compared to CAS+SCMA-70%; whereas they had similar formation of (*E,E*)-2,4-heptadienal and *(E,Z)*-2,6-nonadienal at the last day of the storage. Least formation of primary and secondary oxidation products was observed for emulsions produced with only CAS; however, these emulsions had creaming (5-33%), which limited the interaction between prooxidants in the water phase and lipids. Therefore, it is worth noting that emulsions showed no creaming

(Fig. 2) were oxidatively less stable due to more contact of oil droplet surface with prooxidants existing in the water phase such as metal ions. Another reason could be that the formed cream layer had an increased viscosity, which may hinder the diffusion of reactants (Genot, Meynier & Riaublanc, 2003). Moreover, the higher viscosity of the emulsions produced with CAS and alginates was expected to lead to a superior oxidative stability, as the movement of prooxidant metal ions in the water phase was expected to be slowed down (Sun et al., 2007).

On the other hand, physically stable emulsions such as CAS+SCMA-70% and CAS+SCMA-60% did not differ significantly from emulsions produced with only CAS for the formation of 1-penten-3-ol, (*Z*)-4-heptenal, (*E,E*)-2,4-heptadienal and (*E,Z*)-2,6-nonadienal (Fig 4). Presumably, this is due to the fact that SCMA has one more carboxyl group in its structure compared to LCMA, which is a metal chelator. Moreover, SCMA is expected to be located more in the water phase than LCMA (see section 3.1.1), which might lead to a superior metal chelating activity in the water phase, whereas LCMA attracted metal ions at the oil-water interface.

3.3. Principal component analysis (PCA)

PCA model was used for combining all results in order to visualize the overall picture by using different data obtained from physical and oxidative stability measurements (Fig. 5). PCA showed that PV and volatile compounds were explained by PC1 (62%). Groups were formed according to emulsifier type along the PC1 axis of the PCA bi-plot which was ranked as CAS, CAS+SCMA, CAS+CA and CAS+LCMA when moving from left to right. This information suggested that oxidation was highly affected by different emulsifier combinations used in

emulsions. Oil content was well explained by PC2; groups were formed according to fish oil content which was decreasing from 70% to 50% with increasing PC2 scores. When moving from upper-left to the lower-right side of the PCA plot, viscosity was increasing and droplet size and creaming were decreasing. These findings suggested that these parameters were affected both from oil content and emulsifier type (as discussed in section 3.1), and were explained by PC1 and PC2, respectively. PC3, PC4 and PC5 were also investigated; however, they did not reveal additional information about the data.

Droplet size and creaming were located close to each other; suggesting that the emulsions with larger droplet sizes were more likely to cream. Also, oil content and viscosity were placed close to each other, confirming that higher oil content increases viscosity of the final emulsion which was in line with the discussion under the section 3.1.3.

Emulsions CAS+LCMA-50, 60, 70% and CAS+CA-70% were located to the right side of the PCA bi-plot and so were the primary and secondary oxidation products. This shows that these emulsions had lower oxidative stability compared to others. On the other hand, emulsions with only CAS, CAS+SCMA, CAS+CA-50% and CAS+CA-60% were located far from primary and secondary oxidation products, which indicated that they were more oxidatively stable. Among these samples, emulsions produced with only CAS were not physically stable in terms of creaming, had larger droplet sizes and lower viscosity (see creaming, droplet size and viscosity loadings in Fig. 5). In contrast, CAS+SCMA-70%, CAS+SCMA-60% and CAS+CA-60% were physically more stable in terms of creaming and there were no significant increase in droplet size during their storage (sections 3.1.2). Within these three emulsions, CAS+SCMA-70% and CAS+CA-60% were located closer to oxidation products compared to

CAS+SCMA-60%. Therefore, CAS+SCMA-60% was the most oxidatively stable emulsion having the smallest surface area which limits the interaction of oil and prooxidants.

4. Conclusion

Emulsions produced with alginates and CAS improved creaming stability compared to emulsions prepared with only CAS. High fish oil concentration also enhanced creaming stability regardless of emulsifier type. Both fish oil content and emulsifier type had significant effect on droplet size, viscosity, protein content in the water phase and peroxide value. As expected, emulsions produced with CAS+LCMA had better physical stability such as having smaller droplets and higher viscosity than the rest of the emulsions. This was due to LCMA`s improved emulsifying capacity by being more surface active due to its long chain. However, oxidative stability of the CAS+LCMA emulsions was low compared to emulsions produced with CAS+SCMA and only CAS, due to the unsaturated carbon chain in LCMA structure, which triggered lipid oxidation. As SCMA had the advantage of having terminal hydroxyl group as an extra metal chelator either in the water phase or at the water-oil interface, emulsions produced with CAS+SCMA showed almost as good oxidative stability as emulsions produced with only CAS. Moreover, CAS+SCMA had improved physical stability as evaluated by its smaller droplet size and lower degree of creaming and higher viscosity compared to CAS emulsions. Therefore, these results show the potential of combining CAS and SCMA for the physical and oxidative stabilization of high fat (60-70%) fish oil-in- water emulsions.

Acknowledgments

We would like to thank Arla Foods Ingredients amba and Dupont for donating sodium caseinate and sodium alginate, respectively. We would also like to thank Maritex A／S, Norway for providing cod liver fish oil. Furthermore, we are grateful to Inge Holmberg and Thi Thu Trang Vu for their skillful work in the laboratory. Finally, we would like to thank the Danish Council for Independent Research Technology and Production Sciences for financing the project Mapping and Characterizing of Lipid Oxidation in Emulsified Systems (MAPOX).

The authors have declared no conflict of interest.

References

Aken G. A. (2006). Polysaccharides in food emulsions. In A. M. Stephen, G. O. Phillips & P. A. Williams (Eds.), Food polysaccharides and their applications (pp. 521-539). Taylor & Francis Group LLC.

Antonov, Y. A., Van Puyvelde, P. & Moldenaers, P. (2004). Interfacial tension of aqueous biopolymer mixtures close to the critical point. *International Journal of Biological Macromolecules, 34*, 29-35.

AOCS Official Method Ce 8-89. (1998) Determination of Tocopherols and Tocotrienols in Vegetable Oils and Fats by HPLC; AOCS: Champaign, IL, USA.

Berton-Carabin, C. C., Ropers, M. & Genot, C. (2014). Lipid oxidation in oil-in-water emulsions: Involvement of the interfacial layer. *Comprehensive reviews in food science and food safety, 13,* 945-977.

Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, *37*, 911–917.

Chen, B., McClements, D. J. & Decker, A. (2010). Role of continuous phase anionic polysaccharides on the oxidative stability of menhaden oil-in-water emulsions. *J. Agric. Food Chem., 58*, 3779-3784.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). 2010. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA J 8, 1461–568.

Falkeborg, M., Cheong, L., Gianfico, C., Sztukiel, K. M., Kristensen, K., Glasius, M., Xu, X. & Guo, Z. (2014). Alginate oligosaccharides: Enzymatic preparation and antioxidant property evaluation. *Food Chemistry*, *164*, 185–194.

Falkeborg, M. & Guo, Z. (2015). Dodecenyl succinylated alginate (DSA) as a novel dual-function emulsifier for improved fish oil-in-water emulsions. *Food Hydrocolloids*, *46*, 10-18.

Falkeborg, M., Paitaida, P., Shu, A. N., Pérez, B. & Guo, Z. (2015). Dodecenyl succinylated alginate as a novel material for encapsulation and hyperactivation of lipases. *Carbohydrate Polymers, 133,* 194-202.

Faraji, H., McClements, D. J. & Decker, E. A. (2004). Role of continuous phase protein on the oxidative stability of fish oil-in-water emulsions. *J. Agric. Food Chem., 52*, 4558–4564.

Fomuso, L. B., Corredig, M. & Akoh, C. C. (2002). Effect of emulsifier on oxidation properties of fish oil-based structured lipid emulsions. *J. Agric. Food Chem., 50*, 2957-2961.

Gallaher, J. J., Hollender, R., Peterson, D. G., Roberts, R. F. & Coupland, J. (2005). Effect of composition and antioxidants on the oxidative stability of fluid milk supplemented with an algae oil emulsion. *Int. Dairy J.*, *15*, 333-341.

Genot, C., Meynier, A. & Riaublanc, A. (2003). Lipid oxidation in emulsions. In A. Kamal-Eldin (Ed.), *Lipid oxidation pathways* (pp.190-244). AOCS Publishing.

García-Moreno, P. J., Guadix, A., Guadix, E. M. & Jacobsen, C. (2016). Physical and oxidative stability of fish oil-in-water emulsions stabilized with fish protein hydrolysates. *Food Chemistry, 203,* 124-135.

Hadnadev, T. D., Dokic, P., Krstonosic, V. & Hadnadev, M. (2013) Influence of oil phase concentration on droplet size distribution and stability of oil-in water emulsions. *Eur. J. Lipid Sci. Technol.*,*115*, 313–321.

Hernandez, E. M. (2011). Processing of omega-3 oils. In E. M. Hernandez & M. Hosokawa (Eds.), *Omega-3 oils applications in functional foods* (pp. 107-128). Illinois: AOCS Press (Chapter 5).

Horn, A. F., Nielsen, N. S., Andersen, U., Søgaard, L. H., Horsewell, A. & Jacobsen, C. (2011). Oxidative stability of 70% fish oil-in-water emulsions: Impact of emulsifiers and pH. *Eur. J. Lipid Sci. Technol.*, *113*, 1243–1257.

Horn, A. F., Nielsen, N. S., Jensen, L. S., Horsewell, A. & Jacobsen, C. (2012). The choice of homogenisation equipment affects lipid oxidation in emulsions. *Food Chemistry*, *134*, 803–810.

Hudson, B. J. F. (1990). *Food antioxidants.* Elsevier Applied Food Science Series.

Jacobsen, C., Meyer, A. S. & Adler-Nissen, J. (1998). Oxidation mechanisms in real food emulsions: Method for separation of mayonnaise by ultracentrifugation. *J. Food Lipids*, *5*, 87-101.

Jacobsen, C., Adler-Nissen, J. & Meyer, A. S. (1999) Effect of ascorbic acid on iron release from the emulsifier interface and on the oxidative flavor deterioration in fish oil enriched mayonnaise. *J. Agric. Food Chem., 47*, 4917-4926.

Let, M. B., Jacobsen, C. & Meyer, A. S. (2007). Lipid oxidation in milk, yoghurt, and salad dressing enriched with neat fish oil or pre-emulsified fish oil. *Journal of Agricultural and Food Chemistry, 55*, 7802-7809.

McClements, D. J. & Decker, E. A. (2000). Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. *Journal of Food Science, 65*, 1270–1282.

McClements, E. A., Decker, E. A. & Weiss, J. (2007). Emulsion-based delivery systems for lipophilic bioactive components. *Journal of food science, 72,* 109-124.

Nichols, P. D., McManus, A., Krail, K., Sinclair, A. J. & Miller, M. (2014). Recent advances in omega-3: Health benefits, sources, products and bioavailability. *Nutrients*, *6*, 3727-3733.

Osborn., H. T. & Akoh, C. C. (2004). Effect of emulsifier type, droplet size, and oil concentration on lipid oxidation in structured lipid-based oil-in-water emulsions. *Food Chemistry, 84*, 451-456.

Pal, R. (1996). Effect of droplet size on the rheology of emulsions, *AIChE J., 42*, 3181-3190.

Pallandre, S., Decker, E. A. & McClements, D. J. (2007) Improvement of stability of oil-in-water emulsions containing caseinate-coated droplets by addition of sodium alginate. *Journal of Food Science*, *72,* Nr.9.

Shantha, N. C. & Decker, E. A. (1994). Rapid, sensitive, iron based spectrophotometric methods for determination of peroxide values of food lipids. *J. AOAC Int.*, *77*, 421–424.

Song, C., Shieh, C., Wu, Y., Kalueff, A., Gaikwad, S. & Su, K. (2016). The role of omega-3 polyunsaturated fatty acids eicosapentaenoic and docosahexaenoic acids in the treatment of major depression and Alzheimer's disease: Acting separately or synergistically? *Progress in Lipid Research*, *62*, 41-54.

Sosa-Herrera, M. G., Lozano-Esquivel, I. E., Ponce de León-Ramírez, Y. R. & Martínez-Padilla, L. P. (2012). Effect of added calcium chloride on the physicochemical and rheological properties of aqueous mixtures of sodium caseinate/sodium alginate and respective oil-in-water emulsions. *Food Hydrocolloids*, *29*, 175-184.

Sørensen, A.-D. M., Baron, C. P., Let, M. B., Brüggemann, D. A., Pedersen, L. R. L. & Jacobsen, C. (2007). Homogenization conditions affect the oxidative stability of fish oil enriched milk emulsions: oxidation linked to changes in protein composition at the oil-water interface. *J. Agric. Food Chem., 55,* 1781-1789.

Sun, C., Gunasekaran, S. & Richards, M. P. (2007). Effect of xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions. *Food Hydrocolloids, 21*, 555-564.

Sun, C. & Gunasekaran, S. (2009). Effects of protein concentration and oil-phase volume fraction on the stability and rheology of menhaden oil-in-water emulsions stabilized by whey protein isolate with xanthan gum. *Food Hydrocolloids, 23,* 165-174.

Swaisgood, H. E. (1992). Proteins. In: Fox, P.F. (Ed.), *Advanced dairy chemistry*. London: Elsevier Applied Science.

Tesch, S. & Schubert, H. (2002). Influence of increasing viscosity of the aqueous phase on the short-term stability of protein stabilized emulsions. *Journal of Food Engineering, 52*, 305-312.

Tong, L. M., Sasaki, S., McClements, D. J. & Decker, E. A. (2000). Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. *J. Agric. Food Chem., 45*, 1473-1478.

Wysoczański, T., Sokoła-Wysoczańska, E., Pékala, J., Lochyński, S., Czyz, K., Bodkowski, R., Herbinger, G., Patkowska-Sokoła, B. & Librowski, T. (2016). Omega-3 fatty acids and their role in central nervous system - A review. *Current Medicinal Chemistry*, *23-8*, 816-831.

Yesiltas, B., García-Moreno, P. J., Sørensen, A.-D. M., Jacobsen, C. (2017). Physical and oxidative stability of high fat fish oil-in-water emulsions stabilized with combinations of sodium caseinate and sodium alginate. *European Journal of Lipid Science and Technology*, *119*, 1600484.

Figure Captions

Table 1. Experimental design showing sample name, emulsifier combination and oil content (%)

Figure 1. Effect of fish oil content and emulsifiers on: a) relative protein content in the aqueous phase, b) droplet size (day 1), and c) apparent viscosity at 20 s^{-1} (day 1)

Figure 2. Creaming index of emulsions during storage. The bars indicate the total creaming over 12 days of storage; whereas, the different grey tones indicate the creaming formed between sampling points

Figure 3. Peroxide values of emulsions during storage

Figure 4. Development of 1-penten-3-ol (ng/g), (*Z*)-4-Heptenal (ng/g), (*E,E*)-2,4-Heptadienal (ng/g), (*E,Z*)-2,6-nonadienal (ng/g) in emulsions during storage

Figure 5. Principal component analysis (PCA) plot

Table 1. Experimental design showing sample name, emulsifier combination and oil content (%)

Emulsion code	Emulsifiers	Fish oil%, w/w of total emulsion
CAS+LCMA-50%	CAS+LCMA (sodium caseinate + long chain modified alginate)	50
CAS+LCMA-60%	CAS+LCMA (sodium caseinate + long chain modified alginate)	60
CAS+LCMA-70%	CAS+LCMA (sodium caseinate + long chain modified alginate)	70
CAS+SCMA-50%	CAS+SCMA (sodium caseinate + short chain modified alginate)	50
CAS+SCMA-60%	CAS+SCMA (sodium caseinate + short chain modified alginate)	60
CAS+SCMA-70%	CAS+SCMA (sodium caseinate + short chain modified alginate)	70
CAS+CA-50%	CAS+CA (sodium caseinate + commercial alginate)	50
CAS+CA-60%	CAS+CA (sodium caseinate + commercial alginate)	60
CAS+CA-70%	CAS+CA (sodium caseinate + commercial alginate)	70
CAS-50%	CAS (sodium caseinate)	50
CAS-60%	CAS (sodium caseinate)	60
CAS-70%	CAS (sodium caseinate)	70

*Total emulsifier content was 1.4% (w/w, of total emulsion) for all emulsions and the ratio of CAS to alginates was 1.2.

Highlights

- Fish oil content and type of emulsifier affected stability of fish o/w emulsions
- Addition of alginate improved stability of fish o/w emulsions stabilized with casein
- Long chain modified alginate showed better emulsifying capacity
- Short chain modified alginate improved physical and oxidative stability of emulsions